## PATENT CLAIMS

Subs 5

AR 11549

1. A modified human TNF $\alpha$  molecule capable of raising neutralizing antibodies towards wild-type human TNF $\alpha$  following administration of said modified TNF $\alpha$  molecule to a human host, wherein at least one peptide fragment of the human TNF $\alpha$  molecule has been substituted by at least one peptide known to contain an immunodominant T cell epitope or a truncated form of said molecule containing an immunodominant epitope and one or both flanking regions of the human TNF $\alpha$  molecule comprising at least one TNF $\alpha$  B cell epitope, wherein the substitution introduces a substantial change in the amino acid sequence of any one of the strands of the front  $\beta$ -sheet, in any one of the connecting loops and/or in any one of the B', I or D strands of the back  $\beta$ -sheet.

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2. A modified human TNFα molecule capable of raising neutralizing antibodies towards wild-type human TNFα following administration of said modified TNFα molecule to a human host, wherein at least one peptide fragment of the human TNFα molecule has been substituted by at least one peptide known to contain an immunodominant T cell epitope or a truncated form of said molecule containing an immunodominant epitope and one or both flanking regions of the human TNFα-molecule comprising at least one TNFα B cell epitope, wherein said modified TNFα molecule is substantially free from TNFα activity.

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3. A modified human TNF $\alpha$  molecule according to claim 2, wherein the modified TNF $\alpha$  molecule, when tested in the L929 bioassay, is substantially free from TNF $\alpha$  activity, and wherein antibodies raised against the modified TNF $\alpha$  molecule in a suitable host significantly inhibit the activity of native TNF $\alpha$  in the L929 bioassay, and/or wherein said antibodies significantly inhibit the binding

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of wild-type human  $TNF\alpha$  to the 55 kD  $TNF\alpha$  receptor 1 ( $TNF\alpha$ -R55) or the to the 75 kD  $TNF\alpha$  receptor ( $TNF\alpha$ -R75).

- 4. A modified human TNF $\alpha$  molecule capable of raising neutralizing antibodies towards wild-type human TNF $\alpha$  following administration of said modified TNF $\alpha$  molecule to a human host, wherein at least one peptide fragment of the human TNF $\alpha$  molecule has been substituted by at least one peptide known to contain an immunodominant T cell epitope or a truncated form of said molecule containing an immunodominant epitope and one or both flanking regions of the human TNF $\alpha$ -molecule comprising at least one TNF $\alpha$  B cell epitope wherein the substitution has been made in regions of the TNF $\alpha$  molecule so as to essentially preserve the  $\beta$ -sheet structure of the B and G strands.
- 15 5. Modified human TNF $\alpha$  molecule according to claims 1-47 wherein the substitution has been made in regions of the TNF $\alpha$  molecule which involves the strands of the front  $\beta$ -sheets and/or the connecting loops so as to essentially preserve the  $\beta$ -sheet structure of any of the strands of the back  $\beta$ -sheet.
  - 6. Modified human TNF $\alpha$  molecule according to claims 1-4; wherein the substitution has been made in regions of the TNF $\alpha$  molecule which involve a segment of the D strand of the back  $\beta$ -sheet.
  - 7. Modified human TNF $\alpha$  molecule according to elaims 1-4, wherein the substitution comprises at least a segment of the H strand of the front  $\beta$ -sheet and of the connecting loop to the I strand, preferably amino acids 132 to 146.
    - 8. Modified human  $TNF\alpha$  molecule according to claims 1-4; wherein the substitution comprises segments of the H and I strands and the entire connecting loop, preferably amino acids 132 to 152

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9. Modified human TNF $\alpha$  molecule according to claims 1-4, wherein the substitution comprises a segment of the D strand, at least a segment of the E strand and the entire connecting loop, preferably amino acids 65 to 79 or 64 to 84.

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10. Modified human TNFa molecule according to claims 1—4, wherein the substitution comprises the entire C' and C strands and a segment of the D strand, preferably amino acids 40 to 60.

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11. Modified human TNF $\alpha$  molecule according to claims 1.

A, wherein the substitution comprises at least a segment of the E strand and of the front  $\beta$ -sheet of one or both of the connecting loops, preferably amino acids 76 to 90.

12. Modified TNFa according to Claims 1-4, having the amino acid sequence shown in SEQ ID NO:8.

13. Modified TNF $\alpha$  according to elaims 1-4, having the amino acid sequence shown in SEQ ID NO:10.

14. Modified TNF $\alpha$  molecule according to claims 1-4, having the amino acid sequence shown in SEQ ID NO:4 or SEQ ID NO:16.

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15. Modified TNF $\alpha$  according to claims 1-4, having the amino acid sequence shown in SEQ ID NO:20.

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16. Modified  $TNF\alpha$  according to claims 1-4, having the amino acid sequence shown in SEQ ID NO:14.

25 C 17. Modified human TNF $\alpha$  molecule according to any of claims 1-11, wherein the inserted T cell epitope is promiscuous and known to be immunogenic in a majority of human HLA class II types.

- Modified human TNF molecule according to claim 17, wherein the epitope is derived from Tetanus toxoid, preferably epitope P2 and/or \$\mathbb{P}30.
- Dimers, oligomers or mult/imers of the modified human TNF $\alpha$  molecule according to any one of claims 1-18.
- 20. An isolated DNA molecule that codes for a modified TNF $\alpha$  molecule according to any one of claims 1-18.
- A vector which comprises the isolated DNA molecule 21. according to claim 20.
- An expression vector which comprises the isolated 10 22. DNA molecule according to claim, 20 operatively linked to an expression control sequence.
  - A host, which is transformed with the expression 23. vector of claim 22.
- A host according to claim 23 which is selected from 24. 15 strains of bacteria, yeast, of other fungi and insect, mammalian or avian cell lines.
  - A method of producing a modified human  $TNF\alpha$  molecule claims 1-18, which comprises according to any growing the host cells of claim 23 under suitable conditions permitting production of the modified  $TNF\alpha$  and recovering the modified TNFa so produced.
  - A modified human TNFα molecule according to any of 26. -claims 1-18 in the form of a fusion protein with an adjuvant molecule, preferably an immunologically active adjuvant, such as GM-CSF, HSP70 or interleukin.
  - A vaccine against TNFα, comprising an immunogenic 27. amount of one, or mdre modified human TNFa molecules according to any of claims 1-18 and optionally a pharmaceu-

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tically acceptable adjuvant, such as aluminium phosphate, aluminium hydroxide, calcium phosphate, muramyl dipeptide or iscom.

- 28. A vaccine according to claim 27 for the prevention or treatment of diseases promoted by TNFα release or activity such as chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel diseases, including Crohn's disease and Colitis Ulcerosa, and cancer, disseminated sclerosis, diabetes, psoriasis, osteoporosis and asthma.
- 29. A vaccine against  $TNF\alpha$  comprising isolated DNA which codes for the modified human  $TNF\alpha$  molecule according to any one of claims 1-18 inserted in a suitable expression vector.
- 30. A vaccine according to claim 29 containing a construct comprising a non-infectious non-integrating DNA sequence encoding a modified TNF $\alpha$  molecule according to any of claims 1-18 operatively linked to a promoter sequence which can control the expression of said DNA sequence in humans, in an amount sufficient that uptake of said construct occurs, and sufficient expression occurs to induce a neutralizing antibody response against TNF $\alpha$ .
- 31. A vaccine according to claim 29, comprising a viral expression vector, such as a retroviral expression vector.
  - 32. A vaccine according to any one of claims 27-31 for oral or parenteral, e.g. subcutaneous, intramuscular or intradermal administration.
  - 33. The use of antibodies raised by administering a vaccine according to any one of claims 27-32, preferably monoclonal antibodies.

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35. A method of testing human body fluids for the presence of TNFα which comprises contacting a composition containing modified TNFα according to any one of claims that with a sample of human body fluid and determining whether said antibodies bind to TNFα in said sample.

36. A diagnostic method for  $TNF\alpha$ -related diseases employing an in vitro immunoassay to detect  $TNF\alpha$  in human body fluids.

of a sandwich assay, ELISA assay or equivalent assay, which can be unamplified or amplified, e.g. using avidin/biotin technology.

38. A method for the treatment or prevention of diseases,

15 the pathophysiology of which is at least partially due to

TNFα release or activity comprising administering to an

animal, including a human being, an effective amount of

at least one modified TNFα molecule according to elding

18 optionally in combination with a suitable adjuvant or

20 carrier molecule.

39. Use of a modified  $TNF\alpha$  molecule for the preparation of a medicament for the treatment or prevention of diseases the pathophysiology of which is at least partially due to  $TNF\alpha$  release or activity.

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